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Characterization of Osseous Tissues by Thermogravimetric and Physical Techniques

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Changes in osseous tissue after injury were studied in monkeys with the use of thermogravimetric analysis (TGA) and mechanical strength measurements. An exponential relationship was found to exist between strength and weight loss for repair tissues 3 to 8 weeks old. The quantitative techniques investigated seem promising for the improved characterization of mineralized tissue.

Reparative changes in injured bone have been described on the basis of gross and microanatomic observations.[1] These observations aided in the following grouping of events in the repair process: clot formation, organization of the clot, formation of fibrous callus, formation of primary bony callus, formation of secondary bony callus, and functional reconstruction of the fractured bone. Such qualitative information alone has not been adequate, however, for a clear comparison of different healing rates that may be related to a number of variables such as dietary variations or administration of drugs.

The purpose of this study was to assess applicability of thermogravimetric and physical techniques for quantitative characterization of changes that occur in osseous tissues after injury.

Materials and Methods

Four young adult rhesus macaque monkeys (2.9 to 4.1 kg) were anesthetized intravenously with sodium pentobarbital (30 mg/kg). A small incision was made along the medial aspect of the tibia, the periosteum was reflected, and plugs of cortical bone were removed with a 4 mm diameter trephine operated at 2,000 rpm under a continuous water spray.[2] Two plugs were removed from each tibia and stored in physiologic saline. The initial plugs, which consisted of "mature" cortical bone, served as controls. Subsequent plugs were excised from the site of injury to provide specimens at repair intervals of one through eight weeks. Seventy-one specimens were used.

Shear strength of the wet specimens was determined by the following modification of the punch method of Taylor and Margetis.[3,4] The punch was 2 mm in diameter with a punch-die clearance of 0.013 mm. The apparatus was mounted in a constant strain type testing machine.* The punch was forced through the specimens by operating the testing machine at a crosshead speed of 0.02 inch/minute.

The specimens then were dried for 72 hours at 110 C to fiducial (constant) weight (5 to 13 mg) and placed in the furnace of a thermogravimetric analyzer.† Weight loss as a function of temperature was recorded during heating from ambient temperature to 1,000 C at a programmed rate of 20 C/minute. To facilitate interpretation of thermogravimetric data, material‡ obtained by ethylenediamine extraction[5] of 10 to 20 mesh boiled and defatted bovine femur shaft§ was comminuted by mortar and pestle

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* Instron Model TTCL Universal Testing Machine, Instron Engineering Corp., Canton, Mass.

† Thermogravimetric Analyzer, Model TGS-1, Perkin-Elmer Corp., Norwalk, Conn.

‡ Prepared by Dr. W. V. Loebenstein, Chemist, Dental Research Section, Institute of Materials Research, National Bureau of Standards, Washington, DC.

§ Armour Research Division, Armour and Company, Union Stock Yards, Chicago, Ill.

and subjected to the same analytic procedure.

Results

Shear strength of initial cortical plugs (controls) was 620 ± 140 kg/cm² (Table). Difficulty encountered in obtaining reliable thickness measurements of soft spongy specimens precluded shear strength determinations for repair tissues less than three weeks old. At weekly intervals of three through eight weeks, repair tissue showed respective values of 100, 120, 220, 280, 300, and 290 kg/cm² with standard deviations from 30 to 60 kg/cm² (Table; Fig 1, curve *A*).

On thermogravimetric analysis, ethylenediamine-extracted bone lost weight at a relatively constant rate to about 600 C (Fig 2, curve *A*). Weight loss was more rapid between 600 and 900 C. Losses were not significant between 900 and 1,000 C. The total weight loss for extracted bone was 8.8%. The control plugs lost a total of 35 ± 2% of their fiducial weights (Fig 2, curve *B*). Weight loss was slight (1.0%) to about 200 C. A large loss (31.0%) occurred between 200 and 600 C, followed by a slight additional decrease in weight (3.3%) between 600 and 900 C. Weight remained essentially constant between 900 and 1,000 C. Typical plots of thermogravimetric data for repair tissue are shown in Figure 3. Total weight loss diminished rapidly during the second week of healing (from 86 to 56%) (Table; Fig 1, curve *B*; Fig 3). Losses decreased gradually to 44% during the subsequent six weeks. At the end of the eight week test period, repair tissue gave off 9% more volatile matter than the control specimens.

TABLE
SHEAR STRENGTH AND WEIGHT LOSS OF OSSEOUS REPAIR TISSUE

Specimen Age (Weeks)	No. of Specimens	Shear Strength (kg/cm²)	Weight Loss (%)
1	10	. . .	86.5 ± 5.7*
2	8	. . .	55.6 ± 6.4
3	7	100 ± 30*	54.7 ± 4.0
4	7	120 ± 40	56.9 ± 4.7
5	6	220 ± 60	50.1 ± 4.7
6	5	280 ± 60	45.0 ± 3.1
7	5	300 ± 50	46.0 ± 1.1
8	8	290 ± 30	43.5 ± 1.0

Note: Values for control specimens obtained at time of injury were shear strength, 620 ± 140 kg/cm², and weight loss, 34.7 ± 2.4%.
* Standard deviation.

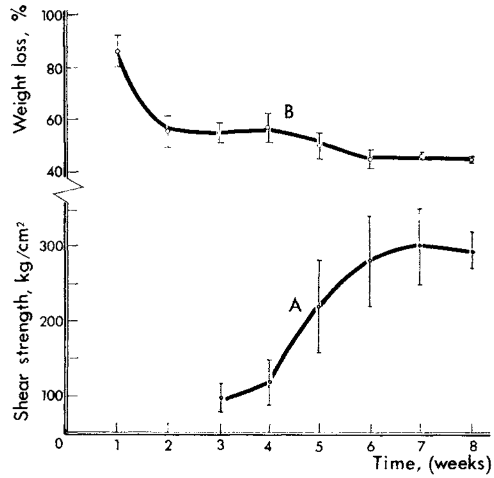

FIG 1.—Strength (*A*) and weight loss (*B*) of repair tissue as function of healing time.

Discussion

Thermal weight losses of osseous tissues are caused by loss of free and adsorbed water, pyrolysis of the organic constituents, and changes in the inorganic phases. In the ethylenediamine-treated bone, a gradual loss of residual free, adsorbed, and bound water was probably the main cause for weight losses to about 500 C (Fig 2, curve *A*). In whole bone, additional losses were caused by the pyrolysis of organic constituents (Fig 2, curve *B*). At temperatures above 600 C,

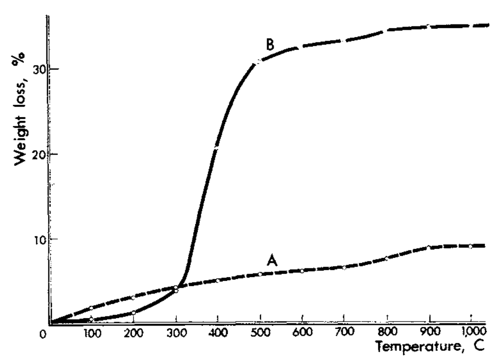

FIG 2.—Weight loss as function of temperature. *A*, Boiled, defatted and ethylenediamine-extracted bovine femur shaft; *B*, initial cortical bone taken from tibias of rhesus macaque monkeys.

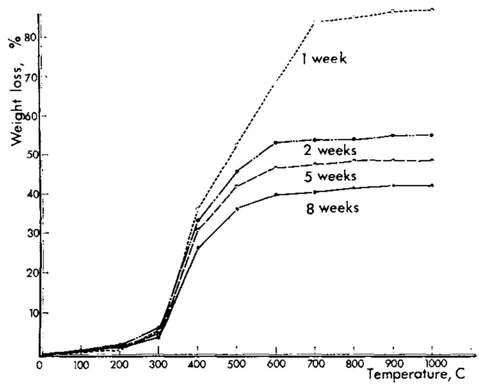

FIG 3.—Weight loss as a function of temperature for repair tissue at indicated healing times.

thermogravimetric plots for extracted and whole bone were nearly parallel and weight losses in both materials probably were caused by similar changes in the inorganic phases. These changes may represent decomposition of small amounts of magnesium and calcium carbonates (700 to 900 C) or the conversion of hydroxyapatite to β-tricalcium phosphate (600 to 800 C) or both.[6,7]

The relationship between strength and total weight loss is illustrated in Figure 4. The curve may be described by the equation. $S = 5{,}975\ e^{(-0.0209)\ (w)}$, where S is shear strength (kg/cm²), e is the natural logarithm base, and w is weight loss (%).

The exponential relationship indicates that small changes in weight loss can be accompanied by large changes in strength. Thus, a 9% higher weight loss may account, in part, for a greater than 50% strength deficit in 8-week-old repair tissue when compared with mature bone (control specimens).

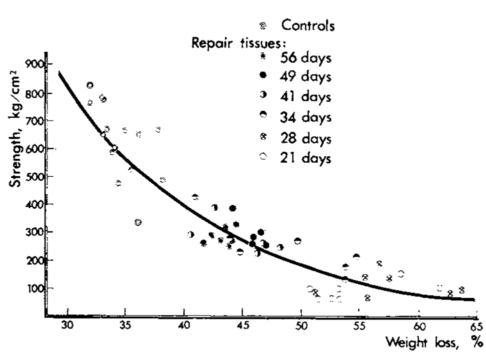

FIG 4.—Relationship between shear strength and weight loss on heating to 1,000 C. Plotted values are for individual specimens.

Both strength and thermogravimetric data correlate with healing stages described by Weinmann and Sicher.[1] The decrease in weight loss between one and two weeks (Fig 1, curve *B*) can be attributed to the organization of the blood clot and the formation of the fibrous and the primary callus. The flat portion on the plot from the second through the fourth week indicates the presence of the primary bony callus. A decrease in weight loss and an increase in strength between four and six weeks probably reflect the formation of the secondary callus. Although repair tissue is not as strong as bone before injury, a healing functional unit may attain nearly the same strength that it had before injury because of increased thickness. The formation of callus and its slow disappearance seem to compensate for the structural deficiencies of repair tissue.

The findings of this study may offer a rapid means for quantitative evaluation of repair rates of osseous tissues. The methods currently are used in conjunction with microscopic techniques in studying effects of zinc salts and chelates on healing rates of experimental bone injuries in monkeys.

Conclusions

Thermogravimetric analysis and shear strength measurements were used to assess compositional and structural changes that occur in osseous tissues after trephine-inflicted injury. Weight losses for repair tissues were 86, 56, 55, 57, 50, 45, 46, and 44% at one through eight weeks, respectively. Shear strength of mature bone specimens (controls) was 620 kg/cm². Repair tissues gave values of 100, 120, 220, 280, 300, and 290 kg/cm² at three through eight weeks. A logarithmic relationship was found to exist between shear strength and weight loss.

The quantitative techniques investigated, in conjunction with gross and microscopic findings, seem promising for the improved characterization of mineralized tissues.

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